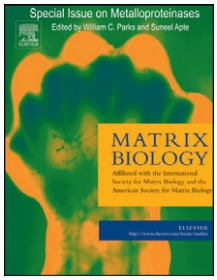
Review



Tumor angiogenesis: MMP-mediated induction of intravasation- and metastasis-sustaining neovasculature

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http://dx.doi.org/10.1016/j.matbio.2015.04.004
Edited by W.C. Parks and S. Apte

Abstract

Metastasis is a distinct stage of cancer progression that requires the development of angiogenic blood vessels serving as conduits for tumor cell dissemination. An accumulated body of evidence indicates that metastasis-supporting neovasculature should possess certain structural characteristics allowing for the process of tumor cell intravasation, an active entry of cancer cells into the vessel interior. It appears that the development of tumor vessels with lumens of a distinctive size and support of these vessels by a discontinuous pericyte coverage constitute critical microarchitectural requirements to: (a) provide accessible points for vessel wall penetration by primary tumor cells; (b) provide enough lumen space for a tumor cell or cell aggregate upon intravasation; and (c) allow for sufficient rate of blood flow to carry away intravasated cells from the primary tumor to the next, proximal or distal site. This review will primarily focus on the functional roles of matrix metalloproteinases (MMPs), which catalytically trigger the development of an intravasation-sustaining neovasculature at the early stages of tumor growth and are also required for the maintenance of a metastasis-supporting state of blood vessels at later stages of cancer progression.

Introduction

The establishment and progression of metastases is a complex multi-step process involving dynamic interactions between cancer cells and their microenvironment [52,78,164]. Within the primary tumor, these reciprocal interactions initiate angiogenic vessel assembly and culminate in the formation of vascular networks contributing to both tumor growth and cancer cell spread to secondary sites [170]. This metastasis-supporting vasculature is shaped in part through continuous proteolytic modifications of the tissue extracellular matrix (ECM) and various cell surface molecules, particularly by the matrix metalloproteinases (MMPs).

The notion that tumor metastasis occurs "due to the conveyance of cells from the primary tumor to some other part of the body, by the blood or lymphatic vessels, where they develop into similar growths" has been acknowledged as early as 1897 [111]. However, it took almost 75 years to suggest that cancer progression could be halted by therapeutic controlling of tumor angiogenesis [60], and another 15 years to conceptualize that the induction of tumor angiogenesis is a critical step in the early development of a solid tumor required for its transition from avascular premalignant to vascular neoplasic stage [61]. By 2000, the induction of tumor angiogenesis by an "angiogenic switch" has become regarded as a hallmark of cancer [76,77]. During that time it has been also established that this angiogenic switch requires a critical angiogenic factor, VEGF, and its proteolytic release from tumor matrix by MMPs, predominantly by the MMP-9 delivered into the tumor microenvironment by tumor-infiltrating leukocytes [12]. Nevertheless, the precise mechanisms whereby MMP-9 and other MMPs induce the development and also sustain those distinct angiogenic vessels capable of supporting tumor cell intravasation and metastatic

dissemination of aggressive cancer cells, are still in the spotlight of cancer research.

Although functionally MMPs have been linked to tissue neovascularization in the early 1990s and multifaceted roles of MMPs in tumor angiogenesis since then are regarded as well established, the literature on how MMPs are mechanistically involved in angiogenic vessel development and angiogenesis-dependent metastasis is still expanding. Thus, when limited to "MMP/Tumor Angiogenesis/Metastasis" as key word criteria, more than 6300 publications are indicated in the PubMed database since 2011 to the present *versus* approximately 3000 publications in the whole decade from 2001 to 2010 and only 30 publications within the 5 years from 1991 to 1995. However, the majority of these "key-words-filtered" MMP publications is centered either on general involvement of MMPs in the neovascularization process or on various aspects of MMP-mediated activation and migration of endothelial cells and not on the unique roles of MMPs in angiogenesis-dependent metastasis. Therefore, we will review almost exclusively the original *in vivo* studies, which illuminate specific mechanisms underlying the MMP-mediated induction and development of a tumor angiogenic vasculature that is functionally and structurally capable of sustaining intravasation and dissemination of cancer cells.

Tumor angiogenic vasculature and tumor cell intravasation

Dysfunctionality of angiogenic vasculature *versus* its sustainability to support tumor cell intravasation and metastasis

According to a generally-accepted notion, angiogenic vessels developing in the primary tumor are structurally abnormal and functionally immature [21]. The tumor vasculature is described as chaotic and torturous, irregular in lumen diameters, dilated and highly permeable, deficient in pericyte coverage and abnormal in endothelial lining [37,64,68]. Nevertheless, this seemingly impaired vasculature is functional enough to provide not only the nutrients and oxygen for a growing tumor, but also the conduits for metastatic dissemination of the escaping tumor cells. Whereas the leakiness and enhanced vessel permeability are compatible with the capacity of angiogenic vasculature to supply nutrients and oxygen to the primary tumor, the overall immature state and dysfunctionality of angiogenic vessels appear to be at odds with the ability of the same vascular networks to sustain active intravasation of tumor cells and their dissemination to secondary sites. Exemplifying such apparent contradiction, the diminishment of pericyte recruitment to primary tumors developing in mice genetically devoid of MMP-9, a critical angiogenic enzyme, has been associated with collapsed morphology of tumor vasculature and substantially inhibited metastasis [24]. Therefore, the pericyte-mediated vessel stabilization [137] and architectural support are essential for the functionality of tumor angiogenic vasculature and its ability to sustain tumor cell dissemination.

Tumor cell intravasation is supported by lumen-containing angiogenic vasculature

While establishing molecular pathways whereby MMPs regulate the process of tumor cell dissemination, we have noticed that high levels of tumor cell intravasation and metastasis frequently correlated with the development and enhanced density within primary tumors of *lumen-containing*, perfusable blood vessels. The presence of non-collapsed, lumen-containing vessels can become an important characteristic of intratumoral vasculature, particularly when the levels of tumor cell intravasation and metastasis are significantly diminished, but the overall density of microvessels or total volume of vasculature have remained unchanged [8,95]. From a mechanistic point, lumen space and blood flow represent obvious requirements for an intravasating tumor cell to enter the angiogenic vessel and be carried away to a secondary site. Therefore, the development of a network of anastomosed angiogenic vessels containing circulating blood and possessing a certain level of blood pressure would likely be prerequisites for the process of metastatic dissemination. In this regard, the vessel-covering pericytes would structurally support the newly-developing tumor vessels and prevent their collapse, allowing for both adequate lumen space to accommodate the size and volume of an intravasating tumor cell or tumor cell aggregates, and sufficient blood flow to carry away the intravasated cells. Reflecting functional importance of these vascular characteristics, the improved pericyte coverage and blood flow are among the "normalization" responses of tumor-associated vasculature to anti-angiogenic therapies [21,68,93], which can lead to accelerated tumor progression and enhanced distant metastasis [50,110,126,145,154].

Functional interplay of MMPs and VEGF in the induction of angiogenic vessels and their maintenance during tumor development

VEGF as a critical molecule governing the development and microarchitecture of angiogenic vessel networks

Numerous molecular pathways and systems have directly or indirectly been implicated in the induction